logic response. However, H-Y serologic response is strongly associated with cGVHD in a multivariable logistic model ($p = 0.007$). These results support the hypothesis that B cell responses to H-Y mHA play an important role in the pathogenesis of cGVHD and suggests that anti-B cell therapy may be useful in the treatment of this disease.

**Table.** *Antibody Response to H-Y Antigens*

| H-Y antigen: | DBY | UTY | ZFY | RPS4Y | EIF1AY | ≥1 H-Y | ≥1 H-X |
|---|---|---|---|---|---|---|---|
| F→M | 34/79 | 18/79 | 17/79 | 5/79 | 5/79 | 40/79 | 14/79 |
| HSCT | (43%) | (23%) | (22%) | (6%) | (6%) | (51%) | (18%) |
| M→M | | | 4/46 | | | 4/46 | 6/46 |
| HSCT | 0 | 0 | (9%) | 0 | 0 | (9%) | (13%) |
| Normal | 11/70 | 4/70 | 3/70 | 15/70 | 9/70 | 29/70 | 12/70 |
| Females | (16%) | (6%) | (4%) | (21%) | (13%) | (41%) | (17%) |
| Normal | 1/64 | | | 1/64 | 3/64 | 5/64 | 5/64 |
| Males | (2%) | 0 | 0 | (2%) | (5%)% | (8%) | (8%) |

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### CHARACTERIZATION OF THE TUMOR-SPECIFIC ACTIVITY OF WT1 SPECIFIC T CELLS GENERATED IN VITRO FROM NORMAL INDIVIDUALS BY SENSITIZATION WITH WT1-PEPTIDE LOADED AUTOLOGOUS EBV TRANSFORMED B CELLS

*Doubrovina, E.S., Doubrovin, M.M., Shieh, J.H., Bierwiaczonek, A., Heller, G., Pamer, E., O'Reilly, R.J. Memorial Sloan-Kettering Cancer Center, New York, NY*

The Wilms' tumor protein WT1 is over-expressed in most acute and chronic leukemias. Isolated T-cell clones have been reported to recognize WT1 peptides binding by HLA-A0201 and HLA-A2402 and lyse $WT1^+$ leukemic lines. To test WT1 peptide immunogenicity in normal individuals, we sensitized T-cells from 9 HLA-$A0201^+$ and 4 HLA-$A2402^+$ donors with autologous EBV transformed B cells (EBV-BLCL) or cytokine-activated monocytes (CAM), loaded with HLA-A0201-binding WT1 peptides RMFPNAPYL or SLGEQQYSV or the HLA- A2402- binding WT1 peptide RVPGVAPTL. WT1-peptide specific cytotoxic and interferon-gamma producing T-cells were generated from each donor. T-cells sensitized with peptide-loaded EBV-BLCL generated higher numbers of WT1 specific T-cells than peptide loaded CAM. The frequencies of WT1 peptide-specific T-cells measured by binding to peptide-HLA tetramers were similar to those generated against individual immunogenic HLA-A0201 binding EBV peptides. These T-cells specifically lysed $WT1^+$ leukemias ($n = 16$) and solid tumors ($n = 12$) in an HLA-restricted manner, but neither lysed autologous or HLA-sharing normal $CD34^+$ hematopoietic progenitor cells ($n = 12$) nor affected their yield of CFU-GM, BFU-C or CFU-MIX. Following adoptive transfer into SCID mice bearing subcutaneous xenografts of $WT1^+$ and $WT1^-$, HLA-$A0201^+$ leukemias, WT1-peptide-specific T-cells preferentially accumulated in and induce regressions only of $WT1^+$ leukemias . The long-term repeated in vivo scintigraphic imaging of such T cells, transduced to express an HSV-TK/GFP fusion gene, following intravenous infusions of the HSV-TK substrate [$^{131}$I]-FIAU demonstrated progressive accumulation of the T cells over a period of 8 days in tumors, expressing targeted antigen and the HLA restricting allele. These studies demonstrate that T cells specific to the self antigen WT1 can be regularly generated from normal individuals and that these cells exhibit significant anti-tumor activity both in vitro and in vivo. Such cells may prove useful for adoptive therapy of leukemias in man.

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### *EX VIVO* ACTIVATED AND EXPANDED $CD8^+$ T CELLS RECOGNIZE MALIGNANT CELLS THROUGH A TCR INDEPENDENT AND NKG2D DEPENDENT PATHWAY

*Verneris, M.R.[1], Karami, M.[2], Baker, J.[2], Jasawal, A.[1], Negrin, R.S.[2] 1. University of Minnesota, Minneapolis, MN; 2. Stanford University, Stanford, CA*

T cells that are activated by TCR crosslinking antibodies and expanded with high doses of IL-2 attain cytotoxicity against malignant target cells without prior exposure or antigen priming. The mechanism of this MHC-unrestricted cytotoxicity is unknown. NKG2D is a cell surface receptor on both NK and $CD8^+$ T cells. Here we examined *ex vivo* activated and expanded $CD8^+$ T cells to determine if NKG2D plays a role in the recognition and triggering of cytotoxicity against malignant targets. FACS analysis showed that NKG2D could be detected on freshly isolated $CD8^+$ cells and expression is increased after 21 days of culture. To access the functional role of NKG2D on *ex vivo* activated and expanded $CD8^+$ T cells we performed $^{51}$Cr release assays using NKG2D blocking antibodies. Blocking NKG2D attenuated the killing of malignant cell targets by >75%, while control antibodies had no effect. Similarly, blocking NKG2D counter-ligands on malignant targets attenuated cytotoxicity by 50-100%. To confirm and extend these findings, we designed siRNA against NKG2D. 72 hours after siRNA transfection, surface NKG2D was nearly undetectable, while other proteins (TCR or CD3) were unchanged. Cells treated with NKG2D siRNA were not cytotoxic, while those treated with control siRNA had cytotoxicity similar to non-transfected cells. To determine whether NKG2D ligation alone triggered cytotoxicity, we performed redirected killing assays using Fc bearing murine target cells. Cells loaded with NKG2D antibodies, but not isotype control antibodies, triggered cytotoxicity. To further demonstrate NKG2D dependent, TCR independent target recognition, we used FACS purified activated and expanded $CD8^+$ T cells and MHC class I def. targets which can not present peptide antigen to $CD8^+$ T cells. Activated and expanded $CD8^+$ T cells lysed these targets and blocking NKG2D nearly completely inhibited cytolysis. Activated and expanded T cells acquire cytolytic activity at □day 10 of culture, in response to high (300 U/ml), but not low (30 U/ml) concentrations of IL-2. DAP10 is the only known adapter protein to transmit NKG2D signals in humans. We therefore assayed for DAP10 at various time points in culture. DAP10 was not detected until day 10, and only in response to high doses of IL-2. Collectively, these studies show that activated and expanded $CD8^+$ T cells recognize targets through a NKG2D dependent, TCR independent pathway, likely controlled at the level of DAP10 expression in response to IL-2.

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### MODULATION OF TREG CELLS BY EXTRACORPOREAL PHOTOPHERESIS IN CHRONIC GRAFT-VS-HOST DISEASE

*Xie, M., Chan, G., Foss, F. Tufts New England Medical Center, Boston, MA*

While the pathogenesis of chronic graft-vs-host disease has not been fully elucidated, alloreactive T cells, antigen presenting cells (APC) and inflammatory cytokine secretion have been implicated. Extracorporeal photopheresis (ECP) has demonstrated efficacy in patients with steroid-refractory acute and chronic GVHD. While the mechanism of action of ECP in GVHD remains to be fully defined, we have demonstrated that clinical response in patients with extensive, refractory cGVHD was associated with normalization of skewed CD4/CD8 ratios and alterations in dendritic cell and T-cell phenotypes, favoring a DC2/Th2 cytokine profile. These results suggest that ECP may indirectly or directly modulate the function or activation of alloreactive T cells in GVHD. In an attempt to determine the effects of ECP on T cell activation we have examined the expression of negative co-stimulatory molecules on T cells from cGVHD patients treated with ECP. We found significant changes in ICOS (inducible costimulatory molecule), but not PD-l (programmed death gene 1) and CD137 (4-1BB) on T cells during ECP treatment. Of 16 patients studied, we found that clinical response to ECP was associated with an increase of $CD4^+ICOS^+$ cells. $ICOS^+$ T-cells are a strong stimulator of IL-10 production and Th2 differentiation, and we have previously reported that ECP treatment is associated with an increase in IL-10 production by T-helper cells in patients with cGVHD. Populations of $CD4^+CD25^+$ cells have recently been identified as immunoregulatory T cells (Treg), however a recent study of allogeneic stem cell grafts suggests that CD4/CD25 coexpression may